

fractions were eluted with a gradient of 20, 50, 70 and 85% of chloroform in hexane and finally 95% chloroform in hexane. The fractions were evaporated to dryness in a rotary evaporator, the residue dissolved in 10% ethanol in chloroform and chemically tested for momilactone as described. GA₃ induced momilactone synthesis in healthy dark grown coleoptiles as well as in leaf sheaths and markedly stimulated momilactone biosynthesis in treated, infected leaf sheaths and coleoptiles.

Gibberellic acid (GA₃), which is a degraded diterpene⁶, may act as a precursor or gibberellin-mediated enzyme (associated with momilactone biosynthesis) production may account for the elicitation of momilactone synthesis in GA₃-treated, non-inoculated tissues. The mechanism involved in the induction of momilactone synthesis by gibberellin requires further study.

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A HISTOCHEMICAL STUDY OF THE EFFECT OF RMI 12,936 ON THE STEROIDOGENIC ACTIVITY IN THE OVARY OF THE GERBIL, *TATERA INDICA*

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17 β -HYDROXY-7 α -methylandroster-5-en-3-one (RMI 12,936) is believed to be antiprogesterone in its

activity. It inhibits ovulation and causes atrophy of the ovary of the rats and disrupts estrous cycle and induces luteolysis in hamsters¹⁻⁴. In the present study the drug's effect on ovarian steroidogenesis in the gerbil, *Tatera indica hardwickei* (Gray) is examined by histochemical analysis.

Female nonpregnant gerbils, freshly collected around Mysore City, were maintained in the laboratory for a few days. The drug RMI 12,936 (kindly courtesy gift to Dr L. L. Albrecht, Morrell Research Centre, USA) was administered subcutaneously at a dosage of 2 mg/animal/day in 0.2 ml olive oil for 15 days. Another group of gerbils received only the vehicle (olive oil) for the same duration. All these animals were kept under laboratory conditions and were sacrificed 24 hr after the last injection. Ovaries were dissected out free from fat and immediately were frozen at -20°C in a cryostat (IEC). Frozen, air-dried sections were taken at 16 μ m thickness. Δ^5 3 β - and 17 β -hydroxysteroid dehydrogenases (HSDHs) were localized as described by Baillie *et al*⁵. Parallel cryostat sections were incubated for glucose-6-phosphate dehydrogenase (G-6-PDH) and NADH-diaphorase as described by Pearse⁶. Sudan Black B was employed to localize lipids⁶. The pattern of distribution and intensity of enzyme activity were visually assessed on a 4-point scale ranging from nil (-) to intense (+++). (table 1).

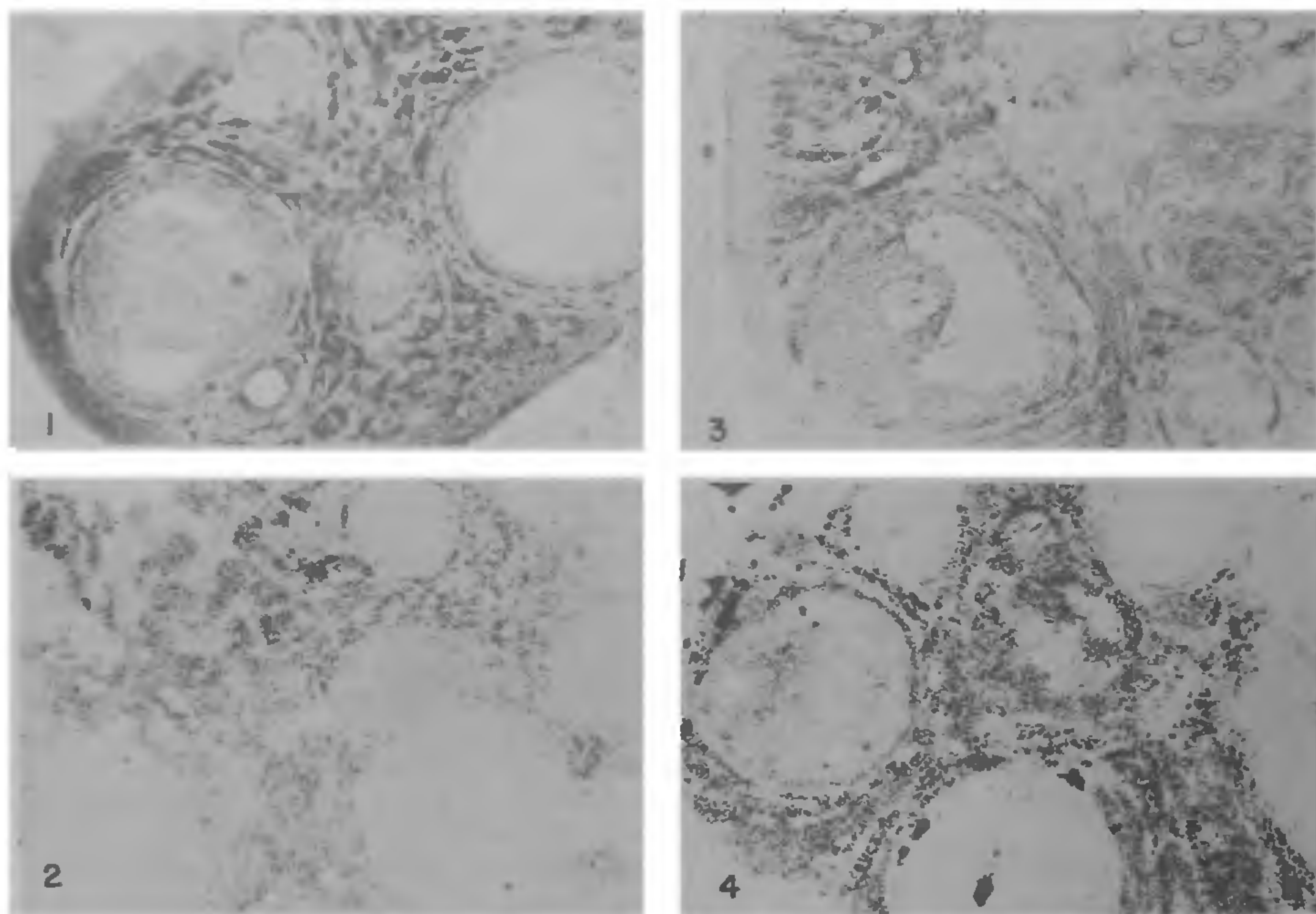
In the control group, HSDHs activity was localized in the theca interna and granulosa cells of the developing follicles, corpus luteum, atretic follicles, ovarian stroma of the ovary (figure 1). The presence of NADH-diaphorase and G-6-PDH was also noted which showed intense reaction in the above sites (table 1). Lipid deposition was observed to be intense in ovarian stroma and moderate in other areas (figure 2). But in the RMI-treated ovary, HSDHs activity was very faint in all the above identified sites of steroidogenic activity (figure 3). NADH-diaphorase and G-6-PDH were moderately active in these regions (table 1). However, intense lipid accumulation was detected in all the regions of the ovary (figure 4).

The histochemical detection of HSDHs indicates the probable sites of steroidogenesis⁵. Likewise the occurrence of G-6-PDH activity in the cells which contain HSDHs provides evidence, though indirect, of the steroidogenic potentiality of these cells, since G-6-PDH is known to provide the NADPH that is needed for hydroxylation during steroidogenesis⁷. Further, the presence of the diaphorase is essential in the localization of HSDHs, as the formazan is deposited by the action of this enzyme at the site of activity. Therefore,

Table 1 Effect of RMI 12,936 on the activity of steroidal enzyme activity and deposition of lipids in the ovary of the gerbil, *Tatera indica*.

Enzymes: Groups:	$\Delta^5\beta$ -HSDH		17β -HSDH		NADH diaphorase		G-6-PDH		Lipids	
	1	2	1	2	1	2	1	2	1	2
Theca interna	a	c	b	c	a	b	a	b	b	a
Follicle cells	b	c	a	c	a	b	a	c	c	b
Corpus luteum	a	b	a	c	a	b	a	c	a	c
Atretic follicles	b	c	b	c	a	c	a	c	b	c
Interstitial tissue (stroma)	a	c	b	c	b	c	b	c	b	c

Pregnenolone and DHA are the substrates used for $\Delta^5\beta$ -HSDH and testosterone & estradiol- 17β for 17β -HSDH. There is no substrate specificity. Groups 1. Control 2. Treated. a-Intense, b-Moderate, c-Faint



Figures 1-4: Cryostat sections of the ovary of control and RMI treated gerbil, *Tatera indica*. $\times 63$. **1.** Localization of $\Delta^5\beta$ -HSDH in the follicles and interstitial tissue of the ovary of control gerbil. **2.** Localization of lipids in the follicles and interstitium of the ovary of control gerbil. **3.** Localization of faint $\Delta^5\beta$ -HSDH activity in the ovary of treated gerbil. **4.** Localization of intense lipid accumulation in the ovary of treated gerbil.

localization of both these enzymes at the same site gives an indirect estimate of steroid dehydrogenase activity⁸. From these points one may safely conclude that the drug RMI 12,936 causes a decrease in steroidogenic activity in the gerbil ovary as evidenced by the obvious decrease in the enzyme activity. The earlier observations are a bit controversial in the sense that Lau and Saksena⁴ found that RMI causes a decrease in the level of estrogen and increase in the level of testosterone and they concluded that a lowered degree of aromatization is the cause for the decreased estrogen level. Hardy *et al*⁹ however, observed that RMI inhibits the biosynthesis of progesterone by being metabolized to a substance believed to be 7 α -methyl testosterone, the metabolite probably being a competitive antagonist of progesterone at the receptor level. In the gerbil, both the HSDHs, which are involved in the conversion of pregnenolone (Δ^5 3 β -HSDH) and interconversion of testosterone and estradiol (17 β -HSDH) seem to be affected by this drug which indicates a general disruption of steroidogenic activity of the ovary and lipid accumulation is also an added evidence to this observation.

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ANNOUNCEMENT

THIRD ANNUAL CONVENTION AND SEMINAR ON 'HYDROLOGY' WITH A COLLOQUIUM ON 'PRECIPITATION AND ANALYSIS AND FLOOD FORECASTING'

The third annual convention and seminar on 'Hydrology' with a colloquium on 'Precipitation Analysis and Flood Forecasting' will be organised at the Indian Institute of Tropical Meteorology, Pune and Department of Geology, University of Poona, Pune, Maharashtra State, India during 5-8 June 1984.

The themes of the Seminar are: (1) Hydro-meteorology and water balance studies, (2) Geomorphology applied to hydrology, (3) Agricultural hydrology, (4) Soil moisture and related studies (5) Engineering hydrology, (6) Forest Hydrology and Urban Hydrology, (7) Geohydrological/Hydrogeological investigations (including studies on aquifer characteristics), (8) Photogeological interpretation and Remote Sensing in Hydrology, (9) Well logging techniques in groundwater hydrology and

case-studies, (10) Geohydrochemistry, water quality and environmental pollution, (11) Geological, geophysical surveys in groundwater exploration, (12) Hydrological model studies, (13) Nuclear hydrology and coastal hydrology, (14) Conjunctive use and management of water resources, (15) Any other related field. Colloquium on 'Precipitation analysis and flood forecasting'.

Venue of the convention: Indian Institute of Tropical Meteorology, Ramdurg House, University Road, Shivajinagar, Pune 411 005.

Further particulars may be had from Prof. Hari-narain, Retd. Director, National Geophysical Research Institute, Chief Co-ordinator, UNDP Projects and President of AHI.